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Muscular Alteration of Gill Geometry *in vitro:* Implications for Bivalve Pumping Processes

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Abstract. In bivalves, water-pumping potential is determined both by ciliary activity and by the geometry of the system of passageways that acts as a conduit for water flow. Smooth muscles intrinsic to the gills of eulamellibranch bivalves possess the anatomical organization needed to regulate the dimensions of these water passageways. The tone of these muscles can be controlled experimentally using excitatory neurotransmitters to elicit muscle contraction and by removing Ca^{++} from the Ringer's solution to induce muscular relaxation. These experimental methods were used to investigate the effects of smooth muscle tone on the gill dimensions of two freshwater bivalves, *Dreissena polymorpha* and *Corbicula fluminea*, and one marine bivalve, *Mercenaria mercenaria*. In addition, endoscopic observations were made from the suprabranchial chamber of a freshwater unionid, *Lampsilis anodontoides*. Contraction of gill muscles led to a significant reduction in interfilament width, internal ostial area, and the cross-sectional area of the water tubes. Endoscopic observation from minimally disturbed *L. anodontoides* revealed rapid constriction of the water tubes upon contraction of the muscles of the gill and gill axis. Taken together, these data support the idea that alteration of smooth muscle tone in the gill provides a mechanism for controlling water-pumping activities.

Introduction

Filter-feeding is a complex process, employed by a diverse assemblage of aquatic animals, in which small particles are separated from the water suspending these food items (reviewed by Jørgensen, 1990; Riisgård and Larsen, 1995). Filter-feeding animals exhibit a variety of convergent designs for pumping water, shaped both by the intrinsic limitations of biological systems and by extrinsic constraints such as those arising from the physical nature of the environment (LaBarbera, 1990; Vogel, 1994). Bivalves, brachiopods, ascidians, and other distinct phyla all use the same type of low-velocity ciliary pump in their filter-feeding processes (LaBarbera, 1990; Vogel, 1994; Riisgård and Larsen, 1995). Most biological pumps consist of a pump and a system, where the pump represents the pressure-generating component and the series of vessels or "pipes" acting as conduits for water flow constitute the system (Jørgensen, 1989; LaBarbera, 1990; Riisgård and Larsen, 1995; Grünbaum *et al.*, 1998). In animals that use low-velocity ciliary pumps, the beating cilia collectively represent the pump, and the system consists of an incurrent region, a transfer region, and an excurrent region (LaBarbera, 1990; Riisgård and Larsen, 1995). The term "bivalve pump" is used to describe the water-pumping processes of filter-feeding bivalves, independent of other events such as particle capture and feeding (Jørgensen *et al.*, 1986).

Extensive research has been carried out to better understand bivalve pumping processes, and data on the rate of water pumping by various bivalves provide an important component needed to develop general models of the pumping process (Foster-Smith, 1976; Møhlenberg and Riisgård, 1979; Silvester and Sleigh, 1984; Meyhöfer, 1985; Jørgensen *et al.*, 1986, 1990; Jørgensen and Riisgård, 1988; Kryger and Riisgård, 1988; Silvester, 1988; Jørgensen, 1989; Jones *et al.*, 1992; Nielsen *et al.*, 1993). There is general agreement that the lateral ciliated cells provide the driving force for water flow and that changes in valve gape and siphon dimension contribute to adjustments in pumping rate. However, little work has addressed the potential role of other system components in regulating pumping processes, and a detailed understanding of the overall control of pumping remains elusive. Debate continues as to whether pump-

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ing and feeding are regulated only in an on/off mode (Jørgensen *et al.*, 1988; Jørgensen, 1996) or whether a more sophisticated physiological regulation of these processes is possible (Ward and Targett, 1989; Wildish and Saulnier, 1993; Navarro *et al.*, 1994; Ward *et al.*, 1997). Further refinement of bivalve pumping models requires more detailed knowledge of the gill itself.

Models of the bivalve pump have relied heavily on the principles of fluid mechanics to develop mathematical descriptions of pumping processes (Foster-Smith, 1976; Silvester and Sleigh, 1984; Jørgensen *et al.*, 1986, 1988; Silvester, 1988; Grünbaum *et al.*, 1998). This type of analysis requires a thorough knowledge of the animal's morphology to accurately estimate pumping properties. For example, the length and width of water passageways are used to estimate pressure losses stemming from the frictional resistance to flow. These system components are generally approached as rigid structures, with models applying fixed estimates of gill geometry to calculate system characteristics (Foster-Smith, 1976; Silvester and Sleigh, 1984; Jørgensen *et al.*, 1986, 1988; Silvester, 1988). Although this is a logical simplification for modeling purposes, a body of evidence indicates that smooth muscles are important in affecting the geometry of the passages that constitute the system (Setna, 1930; Elsey, 1935; Atkins, 1943; Gardiner *et al.*, 1991; Medler and Silverman, 1997, 1998). Poiseuille's law describes fluid flow in a circular pipe as: $Q = \pi \Delta P r^4 / 8 L \mu$, where Q is flow rate, ΔP is pressure difference, r is pipe radius, L is pipe length, and μ is dynamic viscosity of the fluid (LaBarbera, 1990). One of the implications of Poiseuille's law is that relatively small changes in vessel radius result in significant alteration of fluid flow. Many organisms take advantage of this principle to regulate flow by contracting or relaxing smooth muscles or muscle-like cells that line the vessels. For example, vertebrates modify arteriole diameter to regulate blood flow into capillary beds (Eckert *et al.*, 1988), sponges change water flow by contracting or relaxing porocytes and myocytes as water enters the animal (Bagby, 1964; Pearse *et al.*, 1987), and bivalves adjust flow through the alteration of siphon dimensions (Foster-Smith, 1976; Jørgensen *et al.*, 1986, 1988). In this study, we focus on the ability of the eulamellibranch gill to change the dimensions of water passageways by altering the tone of integral smooth muscles.

Bivalves possess several muscles that have the potential to affect water flow. The most widely recognized of these are the adductor muscles and muscles of the mantle edges and siphons that are important for controlling valve gape and siphon dimensions. The integral gill muscles are not as widely appreciated, but are also likely to play a role in basic pumping processes. Each of the individual conduits for water flow is closely associated with smooth muscle fibers that can alter the dimensions of these passageways (Setna, 1930; Elsey, 1935; Atkins, 1943; Gardiner *et al.*, 1991; Medler and Silverman, 1997, 1998). In addition, extensive muscles in the gill axis lying dorsal to the suprabranchial chamber are important in shortening the gill (Setna, 1930; Atkins, 1943). Previous endoscopic studies have documented changes in interfilament width, ostial dimension, and water-tube dimension in living animals (Tankersley and Dimock, 1993; Ward *et al.*, 1994; Tankersley, 1996). Although the exact mechanism of such movements has not been clearly defined, these are consistent with the types of movements observed after the contraction of smooth muscles in the gill (Setna, 1930; Elsey, 1935; Atkins, 1943; Gardiner *et al.*, 1991; Medler and Silverman, 1997). Examination of gills in their fully relaxed and contracted states is useful for defining the extremes in a continuum of gill dimensions. We report here 2- to 6-fold differences between the dimensions of the water passages in fully relaxed and fully contracted gills.

Materials and Methods

Animals

Two freshwater bivalves, *Dreissena polymorpha* and *Corbicula fluminea*, and one marine species, *Mercenaria mercenaria*, were used for gill measurements. Large specimens (shell length about 10 cm) of the freshwater unionid *Lampsilis anodontoides* were used for endoscopic observations. *C. fluminea* and *L. anodontoides* were collected locally from ponds in Baton Rouge, Louisiana. *D. polymorpha* was collected from the Huron River in Michigan, and *M. mercenaria* was purchased from the Marine Biological Laboratory in Woods Hole, Massachusetts.

Solutions

Freshwater animals were maintained in aerated aquaria with artificial pondwater (APW) at 22–24°C as described in Dietz *et al.* (1994). Specimens of *M. mercenaria* were held in aerated aquaria with artificial seawater (ASW) at 4°C as specified in Chambers and De Armendi (1979). Ringer's solutions for the freshwater bivalves were prepared as described for *D. polymorpha* in Dietz *et al.* (1994), with differences in hemolymph osmolality between species being corrected by adding NaCl to the solutions as needed. For *M. mercenaria*, ASW was used in place of Ringer's solution. Calcium-free solutions were prepared by omitting Ca^{++} from the solutions and adding 4 m*M* EDTA in its place (Medler *et al.*, 1999). Neurotransmitters were added to Ringer's solution or ASW at a concentration of 1 m*M*, a pharmacological dose that has been shown to elicit maximal contraction of these muscles (Medler and Silverman, 1997; Gainey *et al.*, 1998). Acetylcholine is effective as an excitatory neurotransmitter in the gills of *D. polymorpha* (Medler and Silverman, 1997) and *C. fluminea* (Medler, unpub. obs.) and was used to stimulate contraction in these species.

Serotonin acts as an excitatory neurotransmitter in the gill muscles of *M. mercenaria* (Gainey *et al.*, 1998) and was used to stimulate contraction in this species.

Gill preparation

Gills were removed from animals by cutting along the dorsal connection to the body with surgical scissors and were placed in the appropriate Ringer's solution or ASW. The gills from one side of an animal were placed in a solution containing Ca^{++} before exposing them to an excitatory neurotransmitter; those of the opposite side were placed in a Ca^{++}-free solution. Interfilament width and internal ostial area were measured from live gills as described below.

Gills normally exhibit severe muscular contraction upon exposure to fixatives such as glutaraldehyde. This was not the case in the present study, since the gills with Ca^{++} available to trigger contraction were already contracted through exposure to excitatory neurotransmitter. In fact, any further contraction that occurred helped to ensure that the gill was in a fully contracted state. We have recently demonstrated that the removal of extracellular Ca^{++} blocks muscle contraction in the gills of *D. polymorpha* (Medler *et al.*, 1999), and this effect is also evident for *C. fluminea* and *M. mercenaria.* Exposure of these relaxed gills to glutaraldehyde failed to initiate any muscular contraction during the fixation process.

Water-tube measurements were made from gill sections. Excised gills were fixed with a 2% glutaraldehyde solution in isosmotic phosphate buffer for the freshwater species and a 2% glutaraldehyde solution in ASW for *M. mercenaria.* Tissues were fixed for 1 h in glutaraldehyde, rinsed twice in either buffer or ASW, and postfixed for 1 h in 1% OsO_4. After fixation, the gills were rinsed twice in deionized water and dehydrated in a graded ethanol series. Whole gills were embedded in LR White (London Resin Co.) medium-grade resin by first placing them in a 1:1 mixture of ethanol and resin for 24 h. They were then transferred to 100% resin for 12 h and embedded flat in fresh resin at 60°C for 24–48 h.

Water-tube dimension

A small portion of the central region of relaxed and contracted gills (approximate midpoint along both the dorsoventral and anterioposterior axes) was cut from embedded, fixed gills and cross-sectioned. Sections were cut using a Reichert-Jung ultracut E ultramicrotome at 1–2 μm thickness and stained with toluidine blue. The sections were viewed with a Nikon Microphot FXA using bright field optics, and the cross-sectional area of the water tube was measured from digitized video images using Image-1 computer software (Universal Imaging Corp.). Because muscle contraction causes a shortening of the gill in an anterioposterior direction, it was necessary to account for this change in our water-tube measurements. This adjustment was made by standardizing water-tube area per unit length of gill (number of filaments spanning the anterioposterior direction). Thus, water-tube area is given as (μm^2/filaments). This correction would be unnecessary if the total cross-sectional area of the gill were measured, but measurements were made from only a portion of the gill. Failing to correct for the shortening would overestimate the cross-sectional area in contracted gills. Water-tube dimensions from relaxed and contracted gills from individuals of each species were compared with paired t tests ($n = 5$).

Studies using fixed and dehydrated tissues are sometimes criticized for introducing artifacts due to shrinkage. Indeed, these procedures do lead to changes in gill dimensions that should be noted when an accurate measure of absolute dimensions is critical (Silverman *et al.*, 1995). In the present study, we were interested only in comparing the relative differences between important dimensions in the fully relaxed and fully contracted states. When the gills were fixed for measurement of water-tube area, each gill pair came from an individual animal and was processed with the same fixatives, dehydration steps, and embedding. Thus, any shrinkage is expected to be proportional in the relaxed and contracted gills, leaving the relative change unaffected.

Interfilament width

Live gills were placed on microscopic slides in the appropriate solution and covered by coverslips elevated on posts of petroleum jelly to prevent the gills from being compressed. Gills were examined with differential interference contrast (DIC) optics on a Nikon Microphot FXA. Interfilament distances from digitized video images were measured as described above, and distances were calibrated with a stage micrometer. Interfilament widths from relaxed and contracted gills from individuals of each species were compared by paired t tests ($n = 5$).

Internal ostial area

Live gills were split into single lamellae and placed in an irrigation chamber as described in a previous study (Medler and Silverman, 1997). Gill lamellae were placed in Ca^{++}-free solutions to completely relax their musculature before any measurements were made and remained in this solution when placed into the chamber. The internal water-channel epithelium was placed toward the bottom of the chamber so that the internal ostia could be observed using an inverted Nikon microscope with Hoffman modulation optics. After the relaxed gill ostia were measured, the chamber was irrigated with Ringer's or ASW containing an excitatory neurotransmitter. The gill was observed and videotaped as it contracted; the ostia were remeasured once contraction was complete (about 1 min later). Ostial areas (μm^2) were measured from digitized video images as described above

and were calibrated with a stage micrometer in the experimental set-up. Ostial areas from relaxed and contracted gills from individuals of each species were compared with paired t tests ($n = 5$).

Scanning electron microscopy

Gills in contracted and relaxed states were fixed as described above. After dehydration, the gills were wrapped in lens paper, critical-point dried, and mounted on stubs. Dehydrated gills were either sectioned or split apart to reveal relevant regions of the gills. Specimens were sputter coated with a mixture of gold and palladium (20 nm) and viewed with a Cambridge S-260 scanning electron microscope. Digitized video images were enhanced for optimal brightness and contrast using Adobe Photoshop 5.0 software (Adobe Systems, Inc.).

Video endoscopy of live gills

An optical insertion tube (OIT) was inserted into the suprabranchial chamber of large specimens of *L. anodontoides* using the general approach described by Tankersley (1996). This species was selected because individuals tend to gape widely, allowing observations to be made without wedging the valves open. Animals were placed in an aerated container of APW (about 4 l) and fixed in position by means of a nylon bolt cemented to one valve. The OIT (1.7 mm diam. × 101 mm long; AEI North America) was attached to a 150 W halogen fiberoptic light source and inserted through the exhalent aperture into the suprabranchial chamber. A mirror sleeve was attached to the OIT to provide the 90° view needed for direct observation of the water tubes. The OIT was attached to a zoom adapter that provided a maximal magnification of about 150×. Maximum resolution was estimated to be approximately 5 μm at maximum magnification. The OIT and zoom adapter were coupled to a Costar color video camera (0.85 cm CCD model CV-730) mounted on a microscope stage. The microscope stage served as a micromanipulator, allowing movements in the X, Y, and Z planes. Observations were recorded on VHS videotape, and digitized video images of portions of these recordings were captured using Image-1 computer software. Images were adjusted for brightness and contrast using Adobe Photoshop 5.0 software.

No pharmacological agents were used during endoscopy. Animals were held in a darkened room, and once the endoscope was positioned, there was minimal disturbance to the animals. Changes in the geometry of the gills and suprabranchial chamber were spontaneous, not resulting from any discernible stimulus.

Results

Water flow through the eulamellibranch gill begins as water moves between parallel filaments and into the external ostia that lead into the water canals of the gill (Fig. 1). These canals empty through internal ostia into the central water channel that separates the ascending and descending gill lamellae. For clarification, some studies use the term "interfilament canal" to mean the space between filaments leading all the way into the central water channel of the gill (Jorgensen *et al.*, 1986), whereas others use the term "ostia" for the same canal system (Foster-Smith, 1976). We use the term "interfilament space" more strictly, as defining the region between filaments on the frontal face of the gill, and the term "ostia" to mean the openings of the water canals that lead from the interfilament space to the central water channel. The central water channel is partitioned into water tubes by septae that connect the two opposing gill lamellae. Water moves dorsally through the water tubes before emptying into the suprabranchial chamber and then out of the excurrent siphon.

Each of the dimensions from gills with relaxed muscles was significantly larger than those with muscles contracted. Interfilament width was approximately 20 μm in relaxed gills from each species and decreased to less than 10 μm after muscle contraction (Fig. 2). Contracted gills had significantly narrower interfilament widths than relaxed gills for each of the three species examined (mean ± SE): *Corbicula fluminea* (23.3 ± 0.7 *vs.* 8.7 ± 1.1 μm; $P < 0.002$), *Dreissena polymorpha* (19.2 ± 2.1 *vs.* 3.3 ± 0.3 μm; $P < 0.001$), *Mercenaria mercenaria* (22.7 ± 1.7 *vs.* 6.5 ± 0.6 μm; $P < 0.001$) (Fig. 2). In fully contracted gills, filaments viewed from the frontal surface appear to abut one another, often producing a zig-zag pattern along the length of the filaments (Fig. 2c). At the level of the lateral ciliated cells, even these gills have about 5 μm between the apical cell surfaces. This apparent discrepancy results from the fact that extended cilia project from the apical cell surfaces and obscure the small gap remaining between filaments.

Internal ostial dimension (per ostium) was quite variable between species, with *C. fluminea* and *M. mercenaria* having larger ostia than *D. polymorpha* (Fig. 3a). After muscular contraction, gills had significantly smaller individual ostial areas for each of the three species examined (mean ± SE): *C. fluminea* (9052 ± 701 *vs.* 3674 ± 404 μm^2; $P < 0.002$), *D. polymorpha* (3175 ± 436 *vs.* 1123 ± 393 μm^2; $P < 0.022$), *M. mercenaria* (16082 ± 1283 *vs.* 9949 ± 1186 μm^2; $P < 0.002$) (Fig. 3).

Water-tube dimension was also variable between species, with larger species having larger gills and water tubes (Fig. 4a). As the muscles of the gill contracted, the cross-sectional area of the water tubes significantly decreased in each species (mean ± SE): *C. fluminea* (8191 ± 727 *vs.* 4164 ± 364 μm^2/filament; $P < 0.002$), *D. polymorpha* (3486 ± 545 *vs.* 502 ± 161 μm^2/filament; $P < 0.005$), *M. mercenaria* (142982 ± 31212 *vs.* 22156 ± 5084 μm^2/filament; $P < 0.001$) (Fig. 4).

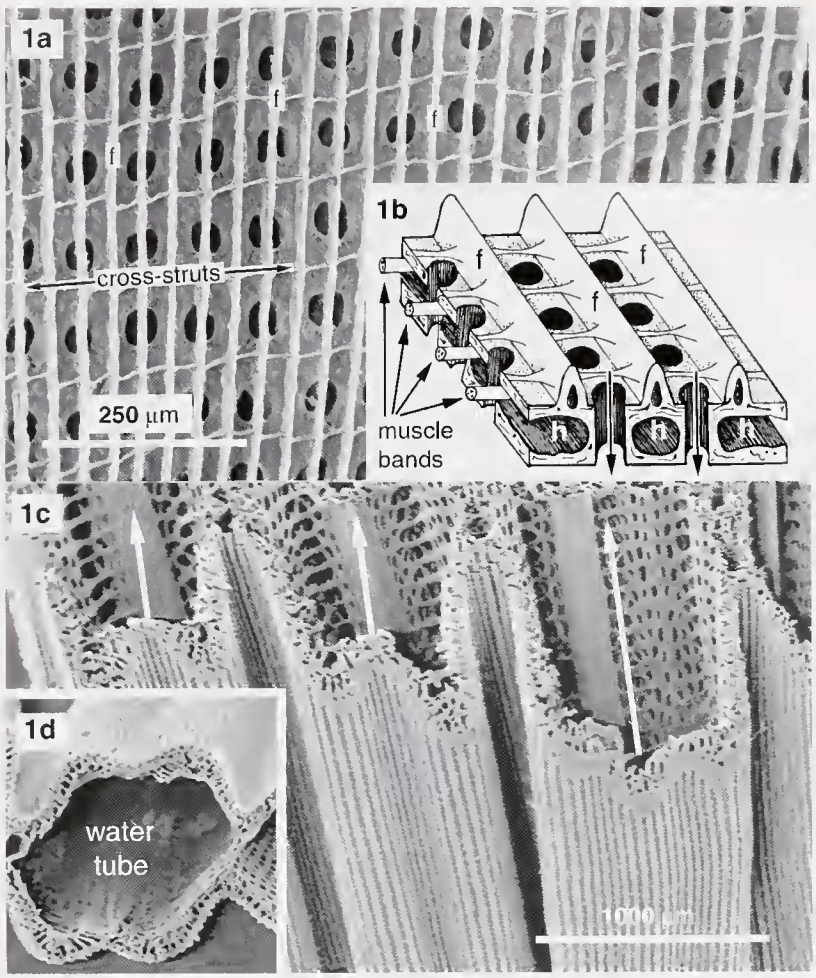

Figure 1. Eulamellibranch gill organization and water flow. (a, b) General organization of a single gill lamella with epithelial tissue removed to reveal the organization of the supporting structures (modified from Medler and Silverman, 1998, with permission from *Invertebrate Biology*). (a) *Dreissena polymorpha* gill (SEM) showing parallel filaments (*f*) held apart by connective tissue cross-struts. Bands of smooth muscles are found below the cross-struts (b); during muscle contraction, the cross-struts bend inwardly and the filaments are drawn closer to one another. In addition to these muscle bands, more diffuse smooth muscle fibers (depicted as thin wavy lines) are found in the connective tissue sheets that enclose the hemocoel (*h*) at the base of the filaments (b). Water is drawn into the central water channel of the gill through water canals (indicated by arrows in b). Ostia (dark openings perforating the gill lamellae) represent the ends of the water canals and provide a route for water to flow into the central region of the gill. (c, d) Frontal (c) and cross-sectional (d) views (SEM) of *Mercenaria mercenaria* gill, demonstrating the movement of water (indicated by arrows in c) through the water tubes of the gill after entering the gill through the water canals. The route of water flow through the gill is between the gill filaments and then through the gill ostia and associated water canals. Water moves to the suprabranchial chamber (not shown) though the water tubes of the central water channel. The suprabranchial chamber at the top of each gill is connected directly to the excurrent aperture where the water leaves the animal.

Endoscopic observations from the suprabranchial chamber of *Lampsilis anodontoides* revealed distinct changes in the geometry of the chamber and water tubes after spontaneous muscle contractions that caused obvious shortening of the gill and suprabranchial chamber. One such change was a rapid reduction in the cross-sectional area of the water tubes, which occurred during a time period of less than 5 s (Fig. 5). Part of the reduction in water-tube area resulted from a shortening of the gill axis, caused by contraction of a large bundle of muscle fibers located dorsal to the suprabranchial chamber and running in the anterioposterior direction. These muscle bundles were described for a number of species by Atkins (1943) and were identified in transverse sections of the dorsal gill region of *L. anodontoides* (data not shown). Many of the observed gill responses were not accompanied by any other obvious changes, such as valve closure. In fact, the valves usually continued to gape widely throughout the observational period.

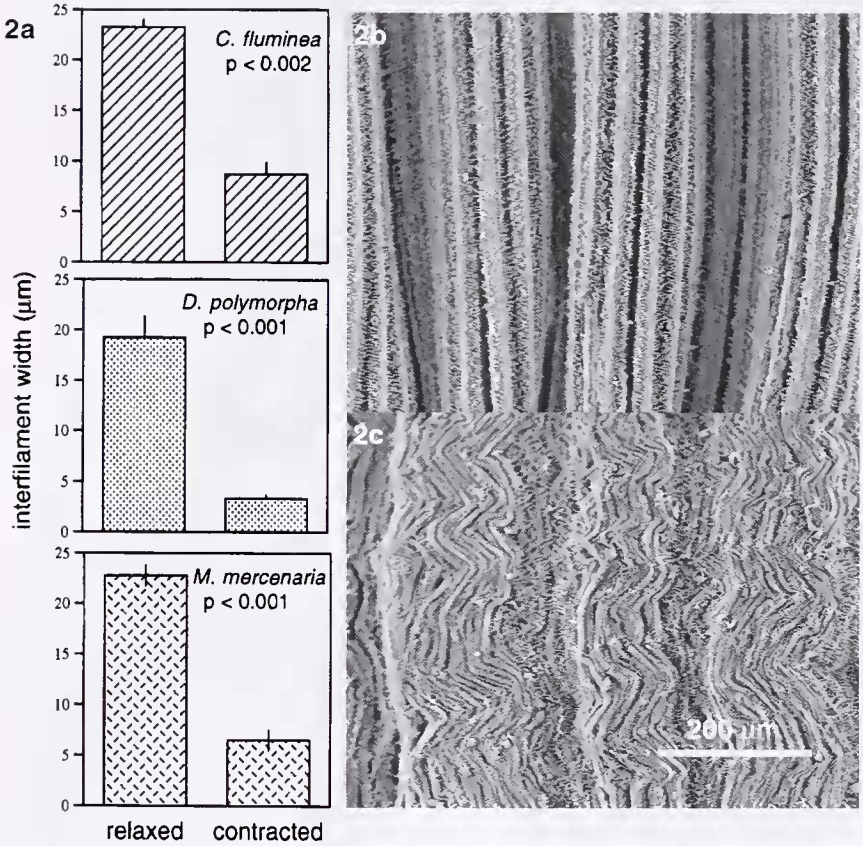

Figure 2. Interfilament distance. (a) Relaxed gills from each of the species (*Corbicula fluminea, Dreissena polymorpha,* and *Mercenaria mercenaria*) had an interfilament distance of about 20 μm, but this distance was significantly reduced to less than 10 μm upon muscle contraction (mean ± SE; $n = 5$). (b, c) Frontal face (SEM) of a *C. fluminea* gill: (b) relaxed gill; (c) gill from the same animal following muscle contraction.

Discussion

Two of the most important factors affecting bivalve pumping potential are the dimensions of the interfilament passages and the exit loss (derived from the kinetic energy carried by the water jet leaving the excurrent siphon) (Foster-Smith, 1976; Jørgensen *et al.*, 1986, 1988; Jørgensen and Riisgård, 1988; Jørgensen, 1989; Riisgård and Larsen, 1995). Both of these factors are ultimately controlled by muscle tone, since integral gill muscles control gill dimensions and exit loss is controlled by the muscles affecting siphon dimensions. It is reasonable to expect higher pumping rates when the gill muscles are fully relaxed, because in this condition the passageways for water flow are significantly more open than when the muscles contract. Jørgensen and colleagues have long held that pumping activities are correlated with the degree of valve gape and the associated changes of the gill (Jørgensen *et al.*, 1986, 1988; Jørgensen and Riisgård, 1988; Jørgensen, 1989, 1990). Their interpretation is that gill dimensions are controlled secondarily to the contraction of muscles within the gill axis and that the muscles of the gill axis contract when the adductor muscles reduce valve gape (Jorgensen *et al.*, 1988; Jørgensen, 1989, 1990). We agree with this general description, but would refine it by stipulating that interfilament distance and other gill dimensions are controlled directly by integral smooth muscles. Part of this distinction relates to the fact that Jørgensen's model is based on animals that possess filibranch gills, rather than the eulamellibranch organization described here. In filibranch gills, there is no direct connection between adjacent filaments aside from that made through the ciliary discs. Nevertheless, these discs clearly cause changes in interfilament distance even in excised gill fragments independent of the gill axis (Jørgensen, 1976; Jones *et al.*, 1992; Medler, unpub. obs.). In addition, such movements are probably aided by the muscles that attach at the base of the ciliary discs in many filibranch species (Atkins, 1943).

Jorgensen and colleagues have highlighted alteration of interfilament distance as a central control mechanism for regulating pumping activities (Jørgensen *et al.*, 1986, 1988; Jørgensen and Riisgård, 1988; Jørgensen, 1989, 1990). There are several possible consequences of changes in

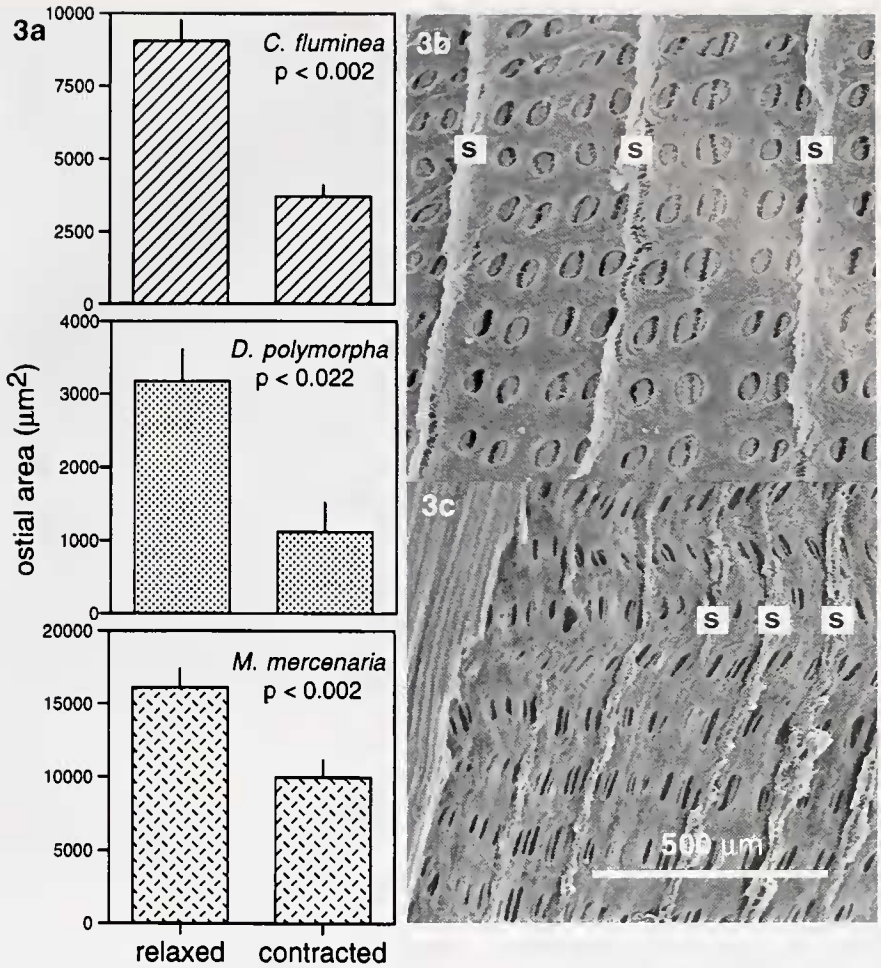

Figure 3. Ostial area. (a) Area of the internal ostia of relaxed gills from each of the species (*Corbicula fluminea, Dreissena polymorpha,* and *Mercenaria mercenaria*) was significantly reduced upon muscular contraction (mean ± SE; $n = 5$). (b, c) Internal face (SEM) of a *D. polymorpha* gill: (b) relaxed gill; (c) gill from the same animal following muscle contraction. The relative position of septa (*s*) that connect opposing lamellae and form the water tubes give an indication of shortening in the anterior to posterior axis.

interfilament distance, with the most obvious being that a change in distance will affect the resistance to water flow (Foster-Smith, 1976; Jorgensen *et al.,* 1986). In addition, it has been suggested that as the filaments move toward one another, the lateral ciliated cells responsible for establishing water flow begin to interfere with one another (Jørgensen *et al.,* 1988; Jørgensen, 1989, 1990). Recent mathematical modeling of gill dimensions has provided further insight into the specific consequences of a particular interfilament distance (Grünbaum *et al.,* 1998). One of the predictions of this model is that the optimal interfilament width for maximizing water flow depends upon the pressure gradient producing the flow. At low pressure differences, a ciliary gap of near 20 μm is optimal; as pressure differences increase, the optimal gap decreases toward 5–10 μm (Grünbaum *et al.,* 1998). This range corresponds well with the distances observed for each of the species in this study (Fig. 2a). When the gill muscles are relaxed, the interfilament width is close to 20 μm, but can quickly change to 10 μm or less as the muscles contract (Medler and Silverman, 1997; Fig. 2a). An environmental variable that may dictate adjustments of pump properties is water temperature, since water viscosity changes inversely with temperature and has direct effects on pumping activities (Jørgensen *et al.,* 1990). It has also been suggested that changes in interfilament width may provide a mechanism to adjust feeding rate and efficiency (Ward *et al.,* 1998). The data in this and previous studies provide evidence that interfilament distance is controlled directly by the activity of smooth muscles, allowing these animals to adjust interfilament distance as needed (Gardiner *et al.,* 1991; Medler and Silverman, 1997).

Connecting the interfilament spaces with the suprabranchial chamber are the passages that constitute the "pipes" of the gill. As indicated by Grünbaum *et al.* (1998),

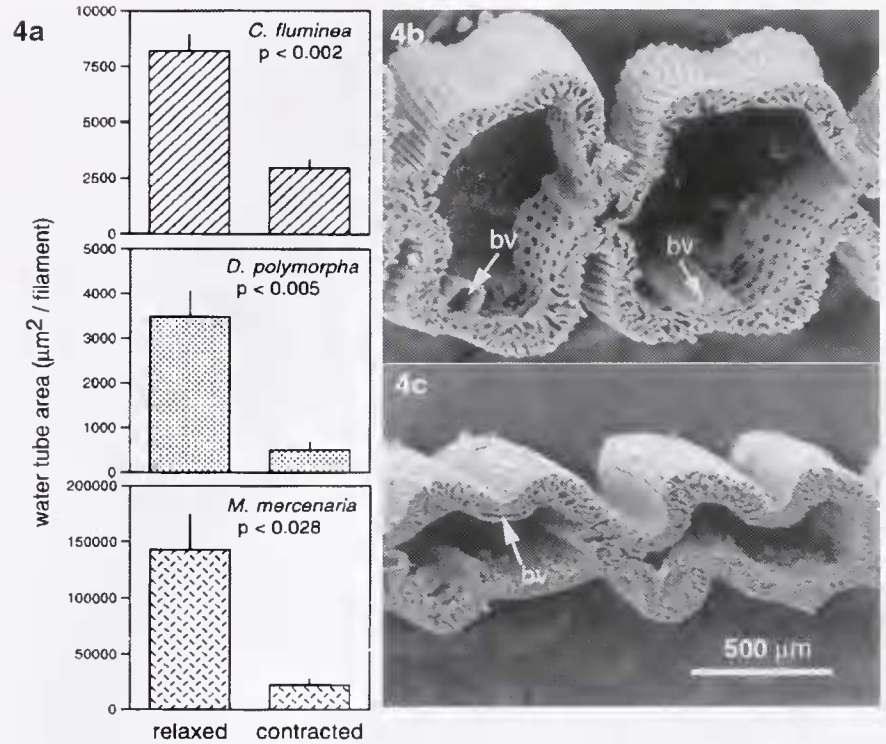

Figure 4. Water-tube dimension. (a) Cross-sectional area of the water tubes per unit gill length significantly decreased upon muscular contraction for each of the species (*Corbicula fluminea, Dreissena polymorpha,* and *Mercenaria mercenaria*) examined (mean ± SE; $n = 5$). (b, c) *M. mercenaria* gill (SEM) in cross-section: (b) relaxed gill; (c) gill from the same animal after the gill muscles contracted. Blood vessels (*bv*) are visible, with only the vessel at the top left appearing to be open.

the pressure drop across the gill filaments is dependent on filament geometry and upon the type of "piping system" to which the filaments are attached. In the eulamellibranch gill, these pipes include the water canals that lead to the central water channel (beginning with external ostia and emptying into the water channel *via* internal ostia) and the water tubes

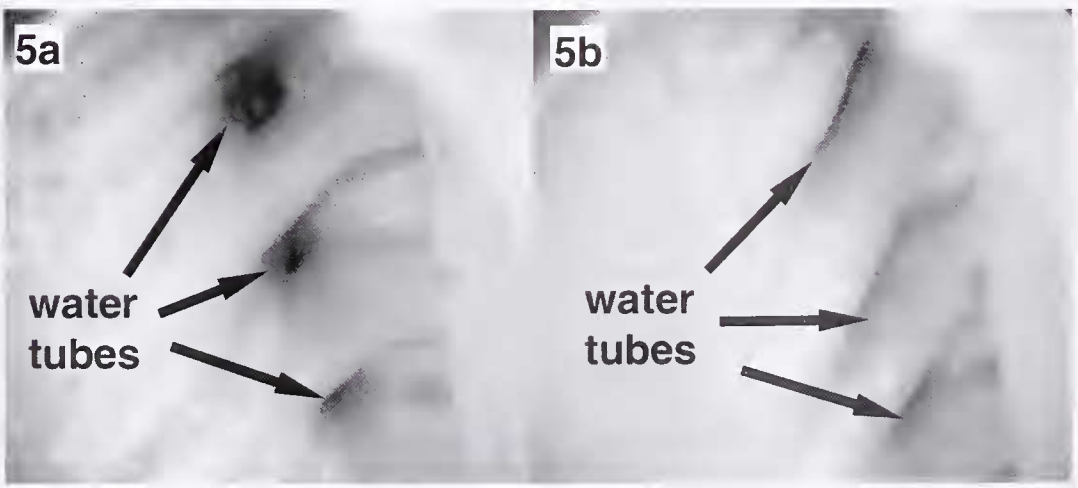

Figure 5. Endoscopic observations. Water tubes are seen at the position where they empty into the suprabranchial chamber of *Lampsilis anodontoides*. During active pumping, the tubes are open (a); when the muscles of the gill and gill axis contract, the water tubes are drawn together and largely close the tubes (b). This type of contraction takes about 1–5 s.

that lead to the suprabranchial chamber. Previous *in vivo* studies from several bivalve species indicate that the ostia are more open during active pumping (Foster-Smith, 1976; Tankersley, 1996), and Jørgensen and colleagues have emphasized a correlation between valve gape, ostial dimension, and rate of water pumping (Jørgensen *et al.*, 1986, 1988; Jorgensen, 1990). Foster-Smith (1976) noted that changes in ostial dimension affect pumping activities, indicating that reductions in ostial size produce pressure losses across the gill. In this and a previous study, we have observed rapid and dramatic changes in internal ostial dimension in live gills (Fig. 3; Medler and Silverman, 1997). In addition, muscle contraction clearly leads to a reduction in the dimension of the water tubes (Figs. 4 and 5). Tankersley (1996) documented similar changes in water-tube dimension in another unionid bivalve, *Pyganodon cataracta*. Although no previous work has addressed the potential effects of such changes in water-tube dimension on pumping processes, Poiseuille's law predicts that as the internal diameter is halved, the flow rate decreases by a factor of 16. Other endoscopic studies have reported a rhythmic expansion and contraction of the gills that was believed to augment water flow through the water tubes (Tankersley and Dimock, 1993; Ward *et al.*, 1994).

Bivalve pumping is a complex process that is dependent on ciliary motors as well as on the muscles that control valve gape, mantle and siphon posture, and gill dimension. The values provided by this study represent the extremes of a range of dimensions that the gills can adopt. It is likely that a continuum of ciliary activity and muscular tone are coordinated through the branchial nerves. Although it is well established that the nerves of the gill have control over ciliary activity (reviewed by Paparo, 1988), almost nothing is understood about the nervous control of the gill muscles. What is known is that the muscles respond to various neurotransmitters *in vitro* (Jørgensen, 1976; Gardiner *et al.*, 1991; Medler and Silverman, 1997; Gainey *et al.*, 1998), and that in a unionid bivalve, the neurotransmitter serotonin induces the gill muscles to relax while increasing the activity of the lateral ciliated cells (Gardiner *et al.*, 1991). The apparent effect of serotonin in this species is to increase pumping rate by enhancing motor activity while simultaneously decreasing the resistance to water flow, but whether these processes are controlled by serotonergic neurons *in vivo* is unknown. Recent work by Gainey *et al.* (1999) reveals that peptides found in nerves in the gills of *Mercenaria mercenaria* have modulatory effects on ciliary activity and that these nerves are closely associated with the muscles of the gills. One of the conclusions drawn from that study is that the peptides are important for modulating both the ciliary and muscular activity involved in feeding activities (Gainey *et al.*, 1999). Understanding the neural control over the muscles of the gill should be a productive focus of future research.

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